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FOREST SERVICE, U.S. DEPT. OF AGRICULTURE, 102 MOTORS AVENUE, UPPER DARBY, PA.

INSECT-INDUCED CRYSTALLIZATION OF WHITE PINE RESINS. I. WHITE-PINE WEEVIL[1]

In breeding programs designed to produce insect-resistant plants, a serious obstacle to progress often is the lack of efficient selection and testing criteria. Natural infestations of some insects are large and severe enough to allow selection of resistant plants directly from the natural plant population. However, the attacks of the white-pine weevil (*Pissodes strobi* Peck), the major pest of eastern white pine (*Pinus strobus* L.), are limited to a small proportion of the trees in a stand or plantation in any given year. Moreover, weevil attack is influenced by the interaction between several environmental factors and the growth rate of the tree. Yet a single successful attack during the first 15 or 20 years of a tree's life may ruin it for quality lumber production. Obviously a definite need exists for criteria by which the relative resistance of white pine trees to this insect can be determined.

Differences in the properties and quantities of the oleoresins produced by pines have long been thought to affect their resistance to certain insect pests. A copious flow of liquid resin seems to function as a defense against those insects that burrow into the tree's tissues. The manner in which such insects cope with these oleoresins is not clearly understood. However, two recent papers point up the possibility that a secretion from the insect may cause the resins to crystallize and thus be inactivated as a deterrent to attack.

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[1] See also: Santamour, Frank S. Jr. INSECT-INDUCED CRYSTALLIZATION OF WHITE PINE RESINS. II. WHITE-PINE CONE BEETLE. U. S. Forest Serv. Res. Note NE-39, 5 pp., 1965.

Harris (1960) collected small drops of vomitus from larvae of the European pine shoot moth (*Rhyacionia buoliana* Schiff.) and mixed this with resin (pine species and source of resin not specified). The mixture of vomitus and resin emulsified readily and hardened into crystals within a few hours. Untreated resin remained sticky for several weeks. Although resin crystallization in different pine species was not compared, it was suggested that the variation in susceptibility to attack may be related to the ease of resin emulsification.

Yates (1962) found that the resin exuded from the tips of cut branches of some pine species could be induced to crystallize by placing live larvae of two *Rhyacionia* species in the resin droplets. The resins of shortleaf and loblolly pines — species susceptible to attack by these tip moths — were readily crystallized, while resins of the resistant longleaf and slash pines showed no crystallization. Control tips of all species remained resinous after 24 hours. Although crystallization normally occurs more rapidly in the susceptible species, it was assumed that the faster-than-normal crystallization on the test tips was caused by the presence of the larvae. Yates stressed the implications of this phenomenon for insect control and the development of insect-resistant strains of pine. A further report on this work (Anonymous 1962) stated that the relationship between insect physiology and resin crystallization had not been determined.

In 1962, a study was begun by the Northeastern Forest Experiment Station to determine the extent to which oleoresin crystallization in various white pine species and hybrids might be induced by larvae of the white-pine weevil.

Materials and Methods

Trees used in this study were selected, mainly on the basis of availability, from ornamental specimens in the Philadelphia area, and from plantations established by the Northeastern Forest Experiment Station at Washington Crossing, New Jersey, and at Williamstown, Massachusetts. The older ornamental trees could not be rated accurately for past weeviling, and the New Jersey plantation was not subject to weevil attack. Therefore data on weeviling were obtained only from trees growing in Massachusetts.

Oleoresin was collected in June, in two ways: from the wood and from branch tips. Wood resin was obtained by drilling 5/8-inch holes slightly upward through the bark into the sapwood, and fitting glass vials into the holes to collect the resin. After 24 hours the vials were removed. Cortical or shoot resin was collected with an attenuated eye-dropper from freshly

cut lateral branches of the current year. All collections were stored in stoppered glass vials at room temperatures, and were tested within 1 to 3 days.

It was assumed that the shoot resin from cortical resin canals of young branches would be more like the resin encountered by larvae in nature than would wood resin. Therefore the two types of resin were kept separate and were tested separately throughout the study.

Larvae of the white-pine weevil were collected from naturally-infested stems of eastern white pine and were utilized while still in a fresh condition. Larvae of other *Pissodes* species and hybrids were obtained from cultures maintained by the Station's Forest Insect Laboratory.

The experimental procedure was as follows. First, a large drop of resin was removed from the vial and placed on a clean glass slide by means of a glass rod. Next, the head and some adjoining thoracic tissue were removed from active larvae by means of a sterilized razor blade. This tissue was then placed in the resin and macerated and mixed with it, using the same glass rod. The slide was then placed in an electric oven at 100° F. and examined at various intervals. A control was used for each test.

In addition, some tests were conducted in which whole unmacerated larvae, larval skin, larvel guts, pupal heads, or fresh frass were used.

Nature and Behavior of Resins

Some background information about the resins themselves is appropriate before discussing the results of the tests with weevil larvae.

Oleoresin can be considered as a supersaturation of rosin in turpentine (Smith 1964). The rosin is composed chiefly of resin acids, and the natural crystallization of oleoresin is the result of precipitating resin acids out of solution. The resin acids are a related group of about 7 to 9 monobasic acids that have the empirical formula $C_{20}H_{30}O_2$. The degree of natural crystallization of oleoresin probably depends on both the total resin content and the proportions of the various acids in the mixture. Baldwin *et al* (1958) stated that crystallization of a given resin acid from rosin (residues left after steam distillation of turpentine) may occur when the resin acid makes up about 30 percent or more of the weight of the rosin. The crystallization of one acid will bring down other resin acids. Knowledge about the qualitative and quantitative relationship of resin acids is limited to the wood oleoresins of the chief turpentine-producing species (longleaf pine and slash pine). No information is available about resin acids in shoot resin. The resin-acid content of pine oleoresins deserves further study.

Natural crystallization.—Crystallization of oleoresin is a natural phenomenon that is expressed in varying degrees in most pine species. Wood resins of some hard pines (subgenus Diploxylon) are particularly precocious in this regard: crystallization may occur in collecting vials during a 24-hour collecting period. However, the wood resins of the soft or white pines seldom exhibit rapid crystallization: of 19 trees of 9 species from which wood resin was collected in this study, the resin from only one, a specimen of *P. parviflora* Seib. & Zucc. (Japanese white pine), showed crystallization during the first week after collection. Resin from another *P. parviflora* did not crystallize during 2 months' storage. Only six wood resins, representing five species, had crystals present after storage for 2 months.

Shoot resins, in contrast, tended to crystallize rapidly in storage. Crystals were observed in most shoot resins within 4 to 12 days after collection. However, no crystals were formed after 1 year in shoot resins of *P. griffithii* McClel. (Himalayan white pine), *P. koraiensis* Sieb. & Zucc. (Korean white pine), *P. peuce* Griseb. (Macedonian white pine) X *griffithii,* and *P. peuce* X *strobus;* and these resins did not react with crushed larval heads. Retarded natural crystallization (2 months) was noted in shoot resins of *P. flexilis* James (limber pine) and *P. ayacahuite* Ehrenb. (Mexican white pine) X *P. griffithii,* both of which showed moderate test crystallization. Thus it appears that the degree of insect-induced crystallization may be related to natural crystallization. Yates (1962) also found that species whose shoot resin crystallized more rapidly in nature gave stronger crystallization reactions under the influence of insects.

Solubility.—All of the wood oleoresins used in this study were completely soluble in methanol, ethanol, and acetone. However, the shoot resins did not all exhibit total solubility. When equal volumes of solvent were added to a sample of shoot resin, a white flocculent material began to settle out. This material did not dissolve completely even when the proportion of solvent to resin was as high as 10 to 1, although some resins were more soluble in acetone than in the other solvents. When the solvent was allowed to evaporate slowly in an open container, the white material redissolved in the resin. The nature of this white material is under investigation.

Monoterpenes.—Preliminary analyses for terpene composition were run by gas chromatographic methods on the shoot and wood resins of some of the trees used in the study. This work was done by Richard H. Smith of the U. S. Forest Service, Pacific Southwest Forest and Range

Table 1.—Monoterpene composition of shoot and wood oleoresins of two pine trees[1]

Component	Pinus strobus		*P. griffithii*	
	Wood	Shoot	Wood	Shoot
	Percent	*Percent*	*Percent*	*Percent*
α—pinene	53.1	58.4	93.8	25.9
β—pinene	42.3	34.9	4.2	36.1
Δ—3—carene	—	—	.9	—
myrcene	1.2	1.0	.7	35.9
limonene	1.2	1.2	.4	—
camphene	1.7	3.5	—	.9
β—phellandrene	.4	1.0	—	1.2

[1] Data supplied by Richard H. Smith, Pacific Southwest Forest and Range Experiment Station, U. S. Forest Service.

Experiment Station, Berkeley, California. Results for one eastern white pine and one Himalayan white pine are given in table 1.

In the eastern white pine, the two types of resin agree fairly closely in monoterpene composition. The shoot resins of four other eastern white pines, two of which were weeviled and two not weeviled, did not differ substantially in composition from the eastern white pine shoot resin. However, in the Himalayan white pine, the shoot resin differed markedly from the wood resin. The possibility that similar differences occur in other species or individuals suggests that published material on the turpentine composition of only the wood oleoresins of pines cannot be used in determining host-pest relationships for insects that primarily attack bark, buds, or perhaps even cones.

Results and Discussion

Crystallization reactions of the resins to the crushed heads or other parts of weevil larvae were observed and rated on a numerical scale after 4 hours. The ratings were as follows:

0—No crystals, or no increase in crystallization over the control.
1—Few crystals; resin clear and sticky.
2—About ¼ crystallized; resin clear and sticky.
3—About ½ crystallized; resin clear and sticky.
4—Some crystals; resins clear, hard, and dry.
5—Crystallization practically complete; resin white, opaque, hard, and dry.

Results of the tests with crushed larval heads in the resins of various white pine species and hybrids are given in table 2. Although not all shoot

Table 2.—Crystallization ratings of white pine resins after 4 hours' contact with crushed heads of weevil larvae

Species or hybrid,[1] *Pinus*—	Resin	
	Wood	Shoot
ayacahuite	0	5
cembra	0	—
flexilis	0	3
griffithii	0	0
koraiensis	0	0
monticola	0[2]	5
parviflora	0[2]	—
peuce	0, 2	5
strobus	0[2]	5[3]
ayacahuite X *strobus*	0	5
ayacahuite X *griffithii*	—	3
griffithii X *parviflora*	0	—
peuce X *griffithii*	—	0
peuce X *strobus*	—	0
strobus X *griffithii*	0	5
strobus X *parviflora*	—	5

[1] In hybrid combinations female parent is listed first.
[2] A few crystals were noted in 1 specimen after 24 hours.
[3] Variation among trees is discussed in the text.

resins crystallize under the test conditions, a much stronger tendency to crystallize was evident among the shoot resins than among the wood resins.[2]

Several of the species and hybrids (table 2) were represented by two or more trees. Responses of individual trees are noted only when the behavior of their resins differed from the general pattern.

Strong crystallization reactions were induced by pupal heads, larval skin, and fresh frass, as well as by larval heads of the weevil. A weak to moderate reaction (rating 2 to 3) was induced by larval guts.

When the whole live larvae were placed in drops of shoot resin of eastern white pine, limited crystallization occurred near the larvae but the resin was still sticky after 12 hours. No crystallization took place in a similar trial with shoot resin of Himalayan white pine.

Larvae of *Pissodes approximatus* Hopk. and of a hybrid weevil, *P. strobi* X *approximatus,* were used in standard tests on eastern white pine resins. Strong crystallization reactions occurred in shoot resin and weak reactions (rating 1 to 2) in wood resin. Larvae of *P. affinis* Rand. induced

[2] In limited tests, the wood resins of *Picea abies* (L.) Karst. (Norway spruce) and *P. asperata* Mast. were readily crystallized.

moderate crystallization (rating 3) in shoot resin and weak crystallization in wood resin of the tree hybrid *P. strobus* X *griffithii.*

Meaningful observations on natural resistance of exotic pine species to our native weevil have been hampered by the limited number of plantings within the optimum range of the insect, and by the imperfectly understood but probably important role that environment and growth rate may play in apparent resistance. Of the two species in which shoot oleoresin was tested and failed to crystallize, Himalayan white pine was considered to be fairly resistant by McAloney (1943) and by Wright and Gabriel (1959). However, Lemmien and Wright (1963) reported that this species was more heavily weeviled than eastern white pine in southern Michigan. *P. koraiensis* was considered to be less susceptible to weevil than eastern white pine (Wright and Gabriel 1959).

Two other species, *P. peuce* and *P. monticola* Dougl. (western white pine), have also been mentioned as possible genetic sources of resistance. Shoot oleoresins of both species were readily crystallized in the presence of crushed heads of weevil larvae. Moderate crystallization occurred in the resins of two trees of *P. flexilis,* a species that McAloney (1943) reported as commonly weeviled and Wright and Gabriel (1959) reported as seldom weeviled.

The lack of resin crystallization for two of the species hybrids deserves special mention. These two trees, both of which were derived from crosses on the same individual *P. peuce* and were growing near each other in Williamstown, Massachusetts, point up the fact that slow- or non-crystallizing resins do not always denote weevil resistance. One of the trees, a specimen of *P. peuce* X *strobus,* showed evidence of past unsuccessful weevil attack and might be considered resistant. The other tree, a hybrid of *P. peuce* X *griffithii,* had been weeviled four times since 1953.

Perhaps the only conclusion to be drawn from both the observational information on weeviling and the resin crystallization tests of exotic species is that much more work is needed. Since it is likely that resistance to weevil is an individual rather than a specific characteristic, large numbers of trees would have to be investigated and tested for resistance.

Shoot resin was collected from 11 eastern white pines in the present study. Eight of these trees were derived from controlled intraspecific crosses among known parents, and three were of unknown parentage. The eight trees were part of a 47-tree plantation of intraspecific hybrids, which have been studied in relation to weevil resistance (Santamour 1964). Six of the 47 trees had not been weeviled. The above-mentioned eight trees included the six unweeviled trees and two others that had been weeviled.

In the tests, both of these weeviled trees and three of the unweeviled ones showed maximum crystallization of shoot resins after 2 hours. The shoot resins of the three trees of unknown parentage, none of which had been weeviled, likewise crystallized readily. However, the other three unweeviled trees among the eight of known parentage gave test reactions rated 0, 1, and 2 after 4 hours and 1, 2, and 4 after 24 hours, thus showing some degree of resistance to insect-induced crystallization of their shoot resins.

The relationship between weevil-induced resin crystallization and known or suspected susceptibility to weevil attack is not clear. Although most of the trees whose resin did not crystallize in the test either had not been weeviled or showed evidence of past unsuccessful weevil attack, the crystallization-resistant *P. peuce* X *griffithii* hybrid was heavily weeviled. When shoot resin from this hybrid was being collected some branches were found that produced very little resin. It may be that the susceptibility of this tree is the result of low resin production under certain conditions. Although the data presented here are somewhat scanty, the study of the crystallization reaction and its causes offers a promising lead for further research.

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— FRANK S. SANTAMOUR JR.[3]

[3] When this study was made, the author was a geneticist on the staff of the Northeastern Forest Experiment Station. At present he is geneticist at the Morris Arboretum, University of Pennsylvania, Philadelphia.